The Structure of certain Chromosomes and the Mechanism of their Division.

By

Arthur Bolles Lee, Hon. F.R.M.S.

With Plates 1 and 2.

Part I. Structure.

(a) *Historical.*

The first suggestion of any structure at all observable in chromosomes seems to be due to Pfitzner ('Morph. Jahrb.', vii, 1882), who suggested that a chromosome is made up of a row of granules of chromatin embedded in an achromatic or less chromatic thread. Belief in these granules—later dignified by the names of 'chromomeres', 'chromioles', and the like—long held sway, and still lingers in many minds. I do not think it necessary to enter into a detailed discussion of this view; for I think it is now indubitable that the supposed granules are nothing but the misinterpreted images of twists of the chromosome, or of bulges in it. The figures illustrating this paper afford abundant instances of bulges caused by twists of the chromosomes; and those illustrating my paper on the chromosomes of Paris quadrifolia ('La Cellule', xxviii, 2, 1912, p. 265) of bulges caused by alveoles in them; either of which, if indistinctly seen, may lend themselves to an erroneous interpretation as granules.[1]

At the present time two other theories are in the field: the chromonema theory, and the alveolation theory.

[1] The chromomere theory seems to have been given up even by Flemming, who at one time accepted it. For in his paper, "Neue Beiträge zur Kenntniss der Zelle", II. Th. ('Arch. mikr. Anat.', xxxvii, 1891), whilst discussing the division of chromosomes, no mention is made of the granules, which he had formerly taken to be active agents of the division; and his figures no longer show any such granules, but in many places show instead more than hints of the bulges of a twisted thread.

The chromonema theory conceives of the chromosome as composed (at least at a certain stage) of a continuous filiform chromatic element—often spirally coiled—supported on an achromatic core, or contained in an achromatic cylindrical matrix.

This notion is due to Baranetzky, who in 1880 ('Bot. Zeitung', p. 241) described and figured, in the pollen mother-cells of Tradescantia virginica, a fine chromatic fibre spirally coiled, at the surface of the chromosomes, round an achromatic core.

In 1901 Janssens ('La Cellule', t. xix, pp. 55 and 58) described similar chromatic spirals uncoiling themselves from the chromatin clumps of the resting spermatogonia of the newt, and even figured similar filaments coiled within the chromosomes of the telophase, closely applied to an enveloping membrane. Later ('La Cellule', t. xxii, 1905, p. 413 and figs. 42 to 50 and 52 to 55) he figured achromatic membranes clearly existing around the 'pachytæne' chromosomes of the auxocytes of Batrachoseps attenuatus, and concluded that in the stages of the bouquet and the strepsinema all the chromosomes are in contact with their neighbours by means of these membranes—'les chromosomes se touchent tous'.

Bonnevie ('Arch. Zellforsch.', i, 1908, p. 450, and particularly pp. 471, 473, 477, 479, 509 ; ii, 1908, p. 201, and particularly pp. 266–70 ; ix, 1913, p. 433) from a study of chromosomes of Ascaris, Allium, and Amphiuma, deduces the following conclusions : A prophasic chromosome consists of an achromatic core on the surface of which is spread a continuous mantle of chromatin (I find no mention of a membrane). In the telophase this mantle becomes differentiated into a spirally coiled thread, whilst the achromatin is cast out into the new nucleus. The spiral threads of chromatin then put forth lateral processes which anastomose with those of neighbouring threads, and so form a nuclear network. At the next prophase the anastomoses are withdrawn, the chromatin threads shorten and thicken, and differentiate into chromosomes showing a newly formed achromatic core with a continuous mantle of chromatin derived from the persisting chromatin of the telophasic spirals. These spirals are therefore the rudiments of a *new generation* of chromosomes.

K. C. *Schneider* (' Festschr. f. R. Hertwig ', i, 1910, p. 215) also describes the chromosomes of the anaphase as consisting of a chromatic spiral enveloping an achromatic core; but finds this spiral become *double* in the telophase. He does not find in the quiescent nucleus a network formed by anastomosing processes of the spirals, but only a tangle formed by the attenuated and elongated spirals themselves. But these spirals are differentiated into chromatic granules united by an (apparently) achromatic thread. The prophasic chromosomes are formed by the condensation of the granules into (two) new chromatic spirals enveloping this thread.

Vejdovsky (' Zum Problem der Vererbungsträger ', Prag, 1912) also finds that a ' ripe ' chromosome consists of an achromatic core round which is wound a chromatic fibre. To this fibre he gives the name of ' chromonema '. He finds no membrane. At the telophase, the achromatic core is cast out, and, swelling, forms the nuclear enchylema. But the chromonema differentiates into a new achromatic thread with chromatic granules (' chromioles ') imbedded in it. The threads thus constituted anastomose into the network of the quiescent nucleus. At the prophase the anastomoses are withdrawn, and the chromioles fuse into a new continuous chromonema, spirally coiled round the persisting threads. In the later prophase the chromonema segments into ' chromomeres ' which undergo bipartition, and so bring about the division of the chromosomes. So that Vejdovsky, though a supporter of the chromonema theory in so far as he recognizes the chromatic thread as a chief constituent of the chromosome, does not entirely discard the granule theory of Balbiani and Pfitzner. Like Bonnevie, he conceives of the chromonemas as the rudiments (*Anlagen*) of a new generation of chromosomes (op. cit., p. 171, *et passim*).

The alveolation theory was foreshadowed by some observations of *van Beneden's*, but has only been worked up into a theory of the quiescent nucleus lately, by Grégoire and his pupils (Grégoire et Wygaerts, "La Reconstitution du Noyau et la Formation des Chromosomes", 'La Cellule', xxi, 1903, p. 7; Grégoire, "La structure de l'élément chromosomique au repos et en division", ibid., xxiii, 1905, p. 311; and other papers by himself and his pupils). According to this, the homogeneous chromosomes of the prophase become during the telophase honeycombed with numerous vacuoles or alveoles, which end by splitting each of them up into a mere network of chromatin. These networks then anastomose by lateral processes, and there is thus formed a network of networks, the reticulum of the quiescent nucleus. At the next prophase the anastomoses are drawn in, and homogeneous chromosomes are formed anew from the remaining reticular tracks by the obliteration of their alveoles and condensation of their honeycombed chromatin into a homogeneous thread.

I have already ('La Cellule', xxviii, 1913, p. 265) published a study of the essential points at issue between Grégoire and Bonnevie, as exemplified in the pollen grains of Paris quadrifolia. I there found the chromosomes to be alveolated as described by Grégoire; but I did not find their alveolation to progress in the telophasic chromosomes to the point of breaking them up into networks. On the contrary, I found their alveoles to disappear, and the chromosomes to condense into thin spiral threads. But I did not find these threads to anastomose into a network in the resting nucleus, as described by Bonnevie. I found nothing worthy of the name of a network, but only a tangle of the much elongated and attenuated spiral chromosomes. I found these persisting throughout the interphase, and at the next prophase forming typical chromosomes by shortening and thickening and at the same time again becoming alveolated. Fig. 1[1] represents a typical group of

[1] This is a drawing of the anaphase shown in fig. 6 of my paper, amended by the addition of the sheath and lateral processes round the axis of the chromosomes, which had escaped me when the original drawing was made. I think it quite likely that there may be also a very fine periaxial spiral, in correspondence with the lateral processes, round the axis of the chromo-

chromosomes honeycombed by easily perceptible alveoles, of the existence of which there can be no doubt. For a detailed description of the characters of these alveoles, the reader will do well to refer to the paper quoted. Fig. 2, which is a slightly corrected copy of fig. 13bis of the same paper, shows the solid spiral threads into which these alveolated chromosomes become transformed during the telophase.

Later, I have extended this study to the chromosomes of the nuclei of the pollen cells and of some tissues of Lilium croceum and L. martagon, and obtained exactly the same results. Combining these results with those of Grégoire and Wygaerts for Trillium grandiflorum and T. cernuum, of Grégoire for Allium cepa, A. ascalonicum, and A. porrum, and of Sharp ('La Cellule', xxix, 1913, p. 297) for Vicia faba, and rejecting as erroneous the statements of those writers who have described in plant chromosomes a spiral fibre instead of alveoles,[1] we find that all the plant chromosomes that have been successfully studied hitherto possess an alveolated structure in the prophases, equatorial phases, and anaphases.

The present paper deals with certain animal chromosomes. Only one recent writer, Kowalski, has described any of these as alveolated. Kowalski ('La Cellule', xxi, 1904, p. 349), studying divers nuclei of the larval Salamander, arrived at the conclusion that their chromosomes all conform to the alveolation theory. I have carefully examined all the chromosomes studied by Kowalski, and many other of the Salamander larva,

somes; and that if this spiral cannot be made out with certainty (I think I sometimes catch glimpses of it), it is because the image of it is obscured by that of the walls of the alveoles. But this, if it exists, is certainly not the spirally coiled thread described by Bonnevie. I intend to return to this point in another paper.

[1] Baranecki's observations may safely be rejected, because they have been controlled by Carnoy and by Strasburger, who did not find the alleged fibre; and those of Bonnevie on Allium, because they are contradicted by the everyday experience of botanical cytologists. Both these writers have apparently misinterpreted images of walls of alveoles, or of torsions of the whole chromosome, as images of a spiral fibre.

and find that neither these nor any other of the animal chromosomes that I have studied do so; but that on the contrary, at one period of their existence, they all do possess a certain spiral differentiation answering, to some extent, to Vejdovsky's 'chromonema'. The following pages set forth the evidence for this, but will, as I think, also show that the advocates of the chromonema theory have pushed it too far; for the spiral differentiation in question does not constitute an independent fibre, and does not form the germ of a new chromosome.

The chromosomes described are chiefly those of prophases, equatorial phases, anaphases, and telophases; but I have touched on those of some interphases in which certain of their characters are demonstrable. I do not attempt in this paper to describe the nuclein elements of completely 'resting' nuclei. The results set forth are based on the study of chromosomes of the Amphibia (chiefly Urodela). Careful investigation of the nuclei of the other classes of the Vertebrata has shown that their chromosomes, though conforming apparently in all respects with those of the Amphibia, are mostly too small to afford trustworthy images of the details in question. The same is the case with most of the Invertebrata, only certain nuclei of the Orthoptera being found to possess chromosomes which, though smaller than most of those of the Amphibia, yet afford images which are often clearer. The majority of the figures are of chromosomes of spermatogonia, the most favourable kind for study. Those of spermatocytes and oöcytes are excluded from the survey, because in them the details are obscured by the complications due to the processes of conjugation. Most of the images described are from paraffin sections: surface preparations show nothing more than these. The most trustworthy fixing agent has been found to be picro-formol (Bouin's formula). Iron haematoxylin has been found to be incomparably the best stain; but it should not be used quite as laid down in the books, which give excessive times and strengths. You should mordant (sections of 7·5 microns, or less) for not more than 2½ minutes in a solution of iron alum of 4 per cent. or weaker; and stain in a half per cent. (or weaker) solution of haematoxylin till the sections appear dark grey, not black (about twenty-five minutes in a virgin solution, or not more than four in one which has already had several slides passed through it); and differentiate in the iron solution for at least a couple of minutes after the sections, examined in water, seem sufficiently extracted. For the stain always appears much lighter in water than in balsam. For the study of the sheath, mount in Gilson's camsal balsam or euparal, rather than in balsam.

(*b*) *Descriptive.*

It will be best to begin with the study of some chromosomes taken at the anaphase, the most favourable moment, figs. 3 to 18.[1] The chromosome of fig. 6, which may be taken as typical, is from a spermatogonium of Salamandra maculosa. It shows the following two (not three) constituents, namely a chromatic (basophilous) axis, and an 'achromatic' (i. e. acidophilous) sheath enveloping this. The chromatic axis is by far the more conspicuous of the two; so much so that, as the sheath is seldom conspicuous enough to compel attention, the axis alone is all that is usually seen, and is therefore generally taken as the whole of the chromosome. But the sheath (which is none other than the achromatic membrane described by Janssens, 'La Cellule', xxii, 1905, p. 413 and figs. 42 to 50 and 52 to 55, as found in the auxocytes of Batrachoseps attenuatus), though it is a difficult object on account of its great tenuity, can generally be made out in well fixed specimens.

The axis has approximately the form of a cylinder, showing a circular section. But it is not a cylinder of regular calibre, for it is generally somewhat dilated at the ends, as seen in figs. 6, 7, 14 (and to a slighter degree in figs. 3 and 4), thus becoming somewhat claviform. And it is generally notably narrower at the polar bend than elsewhere, figs. 3, 4, and

[1] For the objects from which these figs. are taken, see the Explanation of the Plates.

especially 11 ; and at this point is generally somewhat flattened. At its ends (where not sectioned by the knife) it terminates in a smooth dome-shaped surface, from the summit of which there can frequently be seen to emerge a tiny tag, the vestige of its union with its late sister chromosome, figs. 6, 7, 14, 5, 12, all of which show the tag ; and 3 and 4. It is undoubtedly solid, not hollow. Surface views (see the figs. quoted) show no lumen, nor any trace of the alveoles found in plant chromosomes ; but they may seem to show a border darker than the innermost part, as in one or two of the chromosomes of figs. 3, 4, and 5. But in these cases it is generally possible to see that this border is not continuous, but consists of a series of elongated dots. Transverse sections frequently show as disks with a dark border and lighter centre, fig. 15, which may give rise to the impression that there exists an axial lumen. But I have satisfied myself that the axis is in reality solid, and that the dark border is due, for the most part at least, to the periaxial spiral, about to be described, showing there. It is frequently possible, by very careful focusing, to see that this border is darker at one side of the disk than the other, which I take to be due to a sector of the spiral being in sharpest focus there. Thus in fig. 15 *a*, at the top left it is darker to the right ; at the top right, darker at the bottom ; and in the lowest disk darker at the top. And the darker sector can be seen to turn round the disk with every change of focus ; which is just as a spiral viewed end-wise must behave.[1] Similar images are shown, more clearly, by three of the less darkly stained chromosomes of fig. 15 *c*. Those of fig. 15 *b* show the darker border as an apparently entire ring, not a mere sector ; and the fourth chromosome of 15 *c* shows as a disk with a mere hint of a darker border.

Further, in the lighter-coloured centre of the disk there can sometimes be seen a darker comma-shaped dot. One of these is seen as a mere dot in the two upper disks of fig. 15 *a*, and as

[1] For this spiral to be demonstrated it is imperative that the chromosome be not overstained, for if it is the axis will appear as dark as the spiral, and the spiral will not be seen. Vejdovsky's figures grossly exaggerate the distinctness of the spiral at the best of times.

a comma in the lower one. This I have no doubt is nothing but an out-of-focus portion of the periaxial spiral coming into view from a lower depth, in a somewhat tilted chromosome.

I think the utmost that can be admitted in the way of any hollowness of the axis is that this may possibly possess a cortical layer somewhat denser than the rest. But I think the appearances are sufficiently accounted for by the periaxial spiral.

On the surface of this otherwise homogeneous cylinder there runs a spiral of somewhat denser substance than the rest, figs. 3 to 14. This periaxial spiral is evidently somewhat denser than the rest, because it resists decoloration in regressive staining more strongly; but it is evidently of the same composition, for its affinities for stains are the same. It is not something separate from the rest of the cylinder, but is continuous with it. It is not fittingly described as a fibre wound round a core: for there is no space between the spiral and the rest of the axis; there is no hint of a discontinuity between the two either in surface views or in section. Nor should it be described as a fibre countersunk or partially embedded in the axis: for if it were a fibre its section would show as a small circle (or other figure) having a definite limit all round; but these spirals only show a definite limit outside the general surface of the core; inside, they merge in its substance indistinguishably. Vejdovsky's term of 'chromonema' is a misnomer: the thing is not a fibre, but a rib or ridge. It must therefore be taken to be a mere spiral condensation of the cylinder substance.

It is true that cases such as that shown in the left-hand chromosome of fig. 3 are not very infrequent. At the middle of the longer limb of this chromosome there is a break; and the spiral is seen to bridge over the gap between the two parts. But I take it that that is only because its toughness has enabled it to resist where the rest yielded: just as when you break a twig you frequently get the two parts hanging together by a strip of bark.

The periaxial spiral sometimes seems to course uninterruptedly the whole length of the chromosome (with the exception of the extreme tips). But often, as shown in fig. 14, it seems to be interrupted at the polar bend, the bend only showing an attenuated tract of the core without any perceptible ridge on it. At the tips, the spiral ceases at the base of the dome-shaped surface, and is not continued up to its summit, figs. 6, 7, 14.

It seldom shows a regular pitch throughout, for its turns are sometimes very widely spaced, as in figs. 6 and 7, but often so closely approximated that they almost touch one another, as shown at the tip of the right-hand limb of fig. 14. The drawings, in which the spacing between each turn has been reproduced with scrupulous care, will give a better idea of this than any description.

It has been said that the spiral shows no definite limit inside the general surface of the axis; but outside this it does. Its optical section there shows as a series of minute conical elevations, giving, in inferior images, the appearance of a row of minute thorns. These elevations are figured in several of the drawings of recent observers, and are by their authors considered to be in effect minute thorn-like processes. But careful observation of well-preserved specimens (with good objectives and a first-class condenser) shows that the two outlines of each of these apparent cones do not terminate at the apparent apex shown under inferior definition, but merge there into a single line which is continued outwards, generally in a perceptible curve, till it reaches the membranous sheath. And it can often be seen to insert on this by means of a delicate conical enlargement. All the drawings, figs. 2 to 18, show some of these lines, and the enlargement is shown very clearly in figs. 7 and 23, and less clearly, but still recognizably, in several parts of the remaining figures. These enlargements, then, show as a row of minute cones having their bases applied to the inner surface of the sheath, and their apices continuous with the line which springs from the cones on the axis. There is always one of these cones on the sheath for each one on the core. Those on the sheath can often be seen to be situate, not diametrically opposite to those on the core, but a little higher up or lower down, at the extremity of a line which prolongs the course taken by the spiral across the axis. This is shown in fig. 14; but in the remainder of the figures is not shown clearly on account of the frequent derangement of the symmetry of the disposition caused by stretching or other displacement of the sheath. But there can be no doubt that the relations of the two sets of cones are as described.

The line that joins the elevations on the axis to the sheath, including its aponeurosis thereon, is very faint, but it can sometimes be seen to be stained. In that case, it stains in the same tone as the axis; for instance, I have obtained it unmistakably red with safranin. This ligament, then, is a prolongation of the substance of the spiral. And, taking all these facts together, we must come to the conclusion that each of these apparently filiform ligaments is nothing but the optical section of a flange-like or pterygoid membranous expansion of the spiral. This cannot be seen as a membrane, full face, because it winds round the axis in such a way as always to present its edge to the observer; and also because it is so thin (I should think anything under a twentieth of a micron) that if ever a portion of it should come to lie full face it would still be invisible through its thinness.[1]

We may, if we like, call the optical sections of this membrane lateral processes of the axis; which well describes the optical image. But then we must bear in mind that there is in reality only one of them, which courses continuously round the axis like the lamina spiralis cochleae round the modiolus. And we can make a rough model of a chromosome of this type by taking a carpenter's screw and inserting it into a quill into which it will just fit.

The whole of the chromatic axis, the innermost part as well as the spiral and the lateral processes, is most decidedly basophilous: no part of it is achromatic nor acidophilous (which is what the authors quoted in the Introduction mean when

[1] The aponeurosis of this membrane on the sheath can sometimes be seen as a spiral line running along the sheath. I have abstained from drawing it on account of the difficulty of showing it clearly.

they say 'achromatic'). It stains energetically in the fresh state with acid methyl green; and in the fixed state it stains energetically and selectively with safranin, gentian violet, and the other usual basic stains. The only ground that I can discover for the belief in an 'achromatic' core in it is the fact discussed above, that the periaxial spiral generally seems more darkly stained than the rest of the cylinder round which it winds. But that does not in the least point to a difference of chromatophily between the two. The inner part of the axis stains (generally) less darkly than the spiral because it is less dense. And that is all; for the two stain, qualitatively, with exactly the same selectivity for stains.

The sheath is a continuous tubular membrane, of a thickness of the order of about one-twentieth of a micron. It is of irregular calibre, but roughly of a diameter of about three times that of the axis (see figs. 2 to 18 and others). It is very frequently seen to be indented where the lateral processes insert on it, as though it were held down at these points, but blown up between them. It is sometimes seen to be continued round the tip, as in most of the figures given; but sometimes seems only to reach to the base of the dome-like surface, as in fig. 14. It is absolutely structureless. It is decidedly acidophilous, staining readily though somewhat feebly (to about the same degree as spindle fibres, for instance) with Säurefuchsin, Säureviolett, or Lichtgrün; and not staining with basic dyes. The space between this membrane and the axis is filled with a substance of glassy clearness, which is free from all trace of granules or other differentiations, and entirely achromatic, not staining in any way. If it appear to be tinted, as it sometimes may, that is due to the staining of the membrane. This substance may be liquid, or may be gelatinous.

I find the sheath on all anaphase chromosomes of which I can obtain sufficiently good images; and have concluded that it is as universal an attribute of all chromosomes of this stage as the axis and the periaxial spiral.

These, then, are the features which can be detected on favourable specimens of animal chromosomes at the anaphase.

We have now to inquire to what extent they are present in other phases ; and this with special reference to the assertion of Bonnevie and Vejdovsky that at the telophase one part of the chromosome axis is cast out into the new karyoplasm, whilst another persists as a spirally coiled thread which forms the rudiment of the new chromosome.

At the end of the anaphase the 'daughter-star' of chromosomes contracts into a figure which is called by some the 'tassement polaire', a term which we may translate by polar clump. In this clump (figs. 29 to 34) the chromosomes become so densely crowded, and even agglutinated together, that it is impossible to follow out their minute details with accuracy throughout (in the Amphibia : in some other groups the case may be different). Still, enough can be seen in suitably fixed clumps, such as those of figs. 30 and 31, to warrant the assertion that the essential features of the chromosomes persist. In fig. 30, for instance, the chromosome axes can in many places be made out, appearing as thin threads (therefore considerably shrunken) collocated in pairs (an important detail, the discussion of which is best reserved for Part II). The periaxial spirals can just be detected on some of them ; and on others, where they cannot be seen as lines wound round the shaft, their presence is made probable by the lateral processes which can be seen on their edges. And towards the ends of the chromosomes, wherever they stand clear, the sheath membrane can generally be made out as a fine line bridging over the tips of the processes. The sheath can indeed generally be seen round the edges of even highly-agglutinated clumps, figs. 32, 33, 34. In fig. 31 (Bombinator) these details can only just be glimpsed here and there, on account of the smaller size of the elements ; but indubitably exist there as described for fig. 30. We may conclude that at the height of the clump stage the chromosomes—though generally much shrunken, compressed, crumpled, and otherwise distorted—have more or less retained all their essential features.

This stage is of short duration, the clump soon passing by a process of expansion (to be explained in Part II) into the telophase. This next stage will be most conveniently studied in the spermatogonia and oogonia of the Amphibia. For here, as the clump passes into the telophase, it expands into a wide ring, on the surface of which the chromosomes are set on widely spaced meridians, figs. 43, 44, 45, 48, 49, 50, and others. Owing to this arrangement they show only a minimal amount of overlapping, and, standing out on a clear background, can be studied with sufficient accuracy.

In the earliest stages of this process of expansion (figs. 35 to 38) we find much the same state of things as in the denser clump. The paired chromosome axes can be more clearly distinguished : periaxial spirals can be just detected on some of them, and on others their existence is placed beyond all reasonable doubt by the lateral processes visible on the edges of the axes. And the sheath can be made out on many of them (same figs.). In later stages such as figs. 39 to 47, the demonstration of these details becomes more difficult, mainly on account of two complications which here ensue. One of these is the formation of trabeculae (' anastomoses ' of some authors) between the chromosomes. These trabeculae obscure the lateral processes, with which they are easily confused, and so deprive us of an important guide for the detection of the periaxial spirals. The other is, that as the clump expands, the chromosomes elongate ; and as they elongate their duplicate axes twine round one another, figs. 35, 39 to 47.[1] This involves

[1] This gives us the key to Kowalski's assertion (op. cit.) that the chromosomes of the salamander larva are at certain periods alveolated. Thirteen of his figures purport to show the alveoles in question. Eight of these are of telophases. On comparing them with my figs. 39 to 51 it becomes evident at once that Kowalski has interpreted images of doubled and entwined chromosome axes as borders of alveoles—which is very natural, for a thus doubled chromosome easily gives the impression of an alveolated cylinder if you are not able to obtain a sufficiently sharp focusing of its entwined axes. The remaining five of Kowalski's figures of 'alveolated' chromosomes are of spiremes, such as my figs 25 to 27, and manifestly only show that the chromosomes he had before him were double, transverse trabeculae uniting their two moieties being taken for transverse walls of axial cavities in an undivided cylinder or riband.

a continual displacement of the direction of the axes, making it extremely difficult to follow them accurately for more than very short distances, and thus making it next to impossible to distinguish the periaxial spirals running across them. Still, at this stage, it can be inferred with certainty that these exist at least to some extent ; for indubitable lateral processes can be made out in some places ; and the sheath can be observed with certainty in favourable places, as shown in figs. 39 to 45 (in some places of these, where not sufficiently evident in the drawings, I have marked it with a cross).

When the expansion of the clump has attained its greatest extent, we have *the telophasic ring*, figs. 48 to 51, and others. The chromosome axes are here about as distinct as before ; but the periaxial spirals, lateral processes, and sheath seem to be *waning*. The spirals can no longer be seen as lines running across the shaft ; and the lateral processes can only be distinguished from the interchromosomal trabeculae here and there. But this does not necessarily imply that they have diminished in number. For at this stage the chromosomes have elongated considerably ; and since by their elongation the periaxial spirals and their processes must be pulled away from one another, we naturally find far fewer processes than before on any given length of an axis. But this is probably not all that happens. The chromosome of the anaphase and early polar clump is a very *tightly twisted* cylinder ; and there is nothing forced in the supposition that the spirals on its surface, and their lateral processes, are mere effects of the torsion it has undergone. And it appears natural that as the axis elongates at the telophase, it should *untwist* ; and that in consequence of this untwisting the spirals come to subside into the shaft, carrying their processes down with them. Not that the substance of the spirals and processes degenerates or dissolves ; but that it undergoes a change of configuration : as when I extend a finger, wrinkles start up on its surface ; and when I flex it these wrinkles are smoothed down. But be this as it may, it is certain that in the telophase the periaxial spirals and processes begin to wane out of sight, till in the interphase it is seldom possible to detect even a vestige of them with certainty.

As to the sheath at this stage, the appearances are similar. In the nucleus of fig. 47 (Bombinator) (which shows one half of a ring such as that of fig. 50), I am not able to see it, except (possibly) on the chromosome at the extreme left. In the nucleus of fig. 48, a later stage, also Bombinator, I have not been able to detect it. In that of fig. 49 (Triton) I think I can see it in the two places marked with a cross, and glimpse it in one or two others. In that of fig. 50 (Salamandra) I have been able to see it in a fragmentary way in half a dozen places, as marked. In that of fig. 51 (Triton, follicle nucleus of testis) I have been able to detect it in only three places (also marked). It is certainly less abundantly evident in these nuclei than in the earlier stages. And this can hardly be accounted for by greater difficulties in the way of observation ; for the chromosomes are now more widely spaced than before, and observation of their edges should therefore be easier. Add to this that the sheath when detected can only be made out in a fragmentary way ; can only be followed for very short distances ; is less regular than in earlier stages, being frequently distinctly dilated ; and can in some places be seen distinctly to be ruptured (details which it is not possible to render satisfactorily in a drawing). It may be stated as certain that towards the end of the telophase the sheath has generally to a great extent disappeared. And this disappearance seems to be due to a process of real disintegration ending in destruction, rather than to a mere change of configuration or relation of parts. For in completely 'resting' nuclei, even if these are such as to offer every facility for observation, not a trace of it can be detected.

The periaxial spirals and sheath thus lost to view at the telophase come into view again gradually at the next prophase. In the earliest stages in which the spireme is recognizable as being indubitably such (figs. 24 and 25) it seems to consist merely of tortuous naked threads (often clearly double, same figs., and especially fig. 25). These may be united by interchromosomal trabeculae, but show no other lateral processes nor sheath, though they may show in considerable abundance minute nodes or varicosities. And the appearances suggest that these are nothing but nodes of contraction and torsion which may well be the first visible stage of the formation of periaxial spirals and processes. In more advanced stages of the spireme, such as that of fig. 26, lateral processes and a sheath can often be made out with certainty, though with extreme difficulty. At this time (when the loops of the chromosomes are still so closely crowded together that almost all the sheaths are in contact with their neighbours) the lateral processes are sometimes so abundant that when fairly well visible they give the image of a dense network spread over the whole of the ground of the nucleus, as shown in fig. 26. Periaxial spirals cannot be made out on the axes at this time ; but since we have found that lateral processes are signs of the existence of the spirals—being in fact only lateral expansions of these outwards—we must admit that by this time the spirals are in course of formation, if not completely formed, even when we cannot so much as glimpse them.

As the chromosomes contract, they become more widely spaced, and by the time they have contracted into the state known as the ‘ segmented ’ spireme the lateral processes and sheath have come into evidence as clearly as in the anaphase, figs. 27 and 28. In fig. 27 the periaxial spirals cannot be made out, the moieties of the chromosomes being here especially thin (as I invariably find to be the case in endothelium nuclei). In fig. 28 they can just be glimpsed in some places. But not till we come to the chromosomes of the equatorial plate, figs. 19 to 23, do we find the axis clearly differentiated into a shaft with regular spirals on its surface. In equatorial plates whose chromosomes have not entirely assumed the form which they show when definitively arranged on the spindle, the aspect of the axes is still rather that of a structureless though twisted thread than that of a shaft with spirals on it (fig. 19). In the entirely completed and regularized plate the spirals certainly exist throughout, see figs. 20 to 23. If they do not at this time show with all the vigour and distinctness with which they show at the anaphase, this may be sufficiently accounted for by the greater difficulty of observing them in the closely collocated moieties of the equatorial chromosomes. But it may equally well be that they only attain their complete development at the anaphase. We find, then, that the periaxial spirals are only temporary formations. The assertion of Bonnevie and Vejdovsky that they persist after the telophase as rudiments of a new generation of chromosomes is contrary to the facts. For we have found that the chromosomes of the late telophase are for the most part without periaxial spirals and sheath; and that that which persists and passes into the interphase is nothing but the thus simplified axes of the chromosomes. These, on passing into the interphase, frequently become coiled into very regular spirals, such as have been described and figured by many observers (for instance, Bonnevie for Ascaris and Allium, Vejdovsky for Ascaris and other objects, Schneider for Salamandra, and myself for Paris quadrifolia); but these do not consist of periaxial spirals set free from the shaft of the axis, but of the entire axis in a simplified state. The chromonema theory is a mare's nest.

We may now sum up. There are two types of chromosomes: one (hitherto only found in plants) which is alveolated from the prophase to the telophase; and one (hitherto only found in animals) which is not alveolated at those stages or any other. This last consists (at those stages) of a solid basophilous axis, possessing a certain spiral sculpturing of its surface, which we have called the periaxial spiral, and enclosed in an acidophilous sheath. But this sheath is perhaps common to both types; and if the suggestion thrown out in the note on p. 4 should prove correct the periaxial spiral would also be common to both. Then the only important difference between the two would be that the plant chromosomes have an alveolated, i. e. more or less hollow, axis, whilst the animal chromosomes have an entirely solid one.

Part II. Division.

(a) *Historical.*

It was made out by Flemming in 1880 that the chromosomes of the equatorial plate are double, that is, composed of two similar longitudinal halves, closely approximated. The parallelism and close approximation of these halves naturally suggested that they arise by a longitudinal splitting of a previously undivided mother chromosome; and this suggested inquiry as to the means by which the supposed splitting could be brought about.

In 1881 Pfitzner[1] put forth a schema of this splitting which seemed plausible and met with general acceptance. According to this, the mother chromosomes are composed either of a single row of globular granules of chromatin, of a diameter exactly equal to that of the chromosome and embedded in an achromatic matrix; or of a double row of such granules, of only half the size of those of the single row. These double rows are sometimes very closely approximated, sometimes less so; and finally separate from one another as daughter chromosomes. The 'splitting' of the mother chromosome would thus seem to be brought about by the binary division of each of its constituent 'granules'.

This theory won ready acceptance; and the supposed 'granules', under the names of 'Pfitzner's granules', 'microsomes', 'chromomeres', 'chromioles', and the like, are still described and believed in and made the basis of much fanciful explanation.

According to my own very extended observations, this notion of the 'splitting' of chromosomes being brought about by the splitting of their component 'chromomeres' is baseless. For no such granules exist at any time. It is abundantly clear to me that all the appearances that have been described as

[1] "Über den feineren Bau der bei der Zelltheilung auftretenden fadenförmigen Differenzirungen des Zellkerns", in 'Morpholog. Jahrbuch', vii, p. 289—a much quoted but rather wretched performance.

'Pfitzner's granules', 'chromomeres', and the like, are, as already explained, nothing but ill-seen and faultily interpreted images of bulges and twists of the axis of the chromosomes (figs. 3 to 23 and many others of this paper should make this sufficiently clear). It therefore only remains to be seen whether any other mode of division can be made out.

To settle this point, the first step must be to make out at what stage chromosomes can first be seen to be double. According to Flemming ("Neue Beiträge zur Kenntniss der Zelle", ii, in 'Arch. mikr. Anat.', xxxvii, 1891, pp. 737, 744, and 745) the supposed splitting takes place in the spireme stage. And this is apparently the view still taken by the great majority of cytologists.

I am not aware that any observer has asserted a division of chromosomes during the interphase. A longitudinal splitting at the telophase has been asserted by several writers, and with especial insistence by Dehorne. This writer even maintains (in his "Recherches sur la division de la cellule", in 'Arch. f. Zellforschung', vi, 1911, p. 613) that it may take place as far back as the anaphase. This is indubitably erroneous. For beyond all doubt at this stage the chromosomes show no hint of duplicity. But as regards the telophase I find that—in some cases at least—at that stage the chromosomes are certainly double—in a sense; and I acknowledge the essential correctness of Dehorne's clever figs. 7, 9, 10, 11, 12, and 18 (his fig. 6, which corresponds to my fig. 43, I think has been imperfectly understood by him). But I find no trace of any evidence that this duplicity is brought about by a longitudinal splitting.

A division of the chromosomes at the telophase has also been maintained by K. C. Schneider. In his 'Lehrbuch der vergleichenden Histologie', 1902, pp. 10, 118, 848, and 939, he states it as a probable inference. He suggests that at this stage the chromosomes segment transversely at the polar bends; and that the two moieties thus formed grow past one another so as to become parallelly approximated throughout their lengths. I have duly investigated this point, and find no signs of such a process. I need not enter into further details, as Schneider himself seems to have abandoned his supposition. For in a later work (his "Histologische Mittheilungen", iii, "Chromosomengenese", in 'Festschr. f. R. Hertwig', i, 1910, pp. 218, 219, 221) he maintains his view that a division of the chromosomes probably takes place at the telophase (or anaphase), but now supposes it to be a longitudinal one.[1]

Of this also I find no evidence. But I do find evidence of another and simpler process by which the observed images of duplicity are brought about To the consideration of this we may now proceed.

(b) *Descriptive.*

We have already seen incidentally, in Part I, that in the Amphibia the chromosomes of the later telophase are double structures, that is, that they consist of two chromatic threads, longitudinally collocated and more or less entwined.

This is by no means peculiar to the Amphibia. In smaller chromosomes than theirs the images are more difficult; and in much smaller ones it may be impossible to obtain satisfactory resolution. But enough can be made out to leave no doubt that it is a very widespread phenomenon. In the Mammalia I have found it fairly clear in Homo, fig. 54. In some of the Insecta (notably the Orthoptera) it is as certain as in the Amphibia, see figs. 62, 66, 67. I think we may take it as the invariable rule that in animals all the telophase chromosomes are thus doubled, that is, possess already the duplicity observed in the chromosomes of the prophase. This relieves us from the necessity of looking for any process of splitting in the phases between the telophase and the prophase; and it only remains for us to make out in what way the telophasic doubling is brought about.

[1] The reason he gives for this is a strange one. He admits (p. 218) that the daughter chromosomes of the metaphase only show one spiral; but thinks (without asserting it positively) that in the anaphase and telophase they contain two, because 'the coils they show are so closely set that they could hardly be the expression of a single spiral'. How about a reel of cotton ?

To ascertain this we must return to the study of the earlier telophase, or polar clump. In the daughter-star of the anaphase (figs. 3, 4, 5, 61) we have a loose assemblage of chromosomes, radially arranged in a ring. These contract into short staves; and as they contract the whole figure shrinks (figs. 29 to 34), so that the staves become closely huddled together and come into contact by their margins. They generally seem to agglutinate there, and their outlines become hardly distinguishable, indeed very often quite indistinguishable. The clump then appears (figs. 30, 31, 33) as an almost homogeneous ribbed disk, with a central pore, generally obturated by a perforated membrane or web formed (as shown by profile views) by the confluent remains of the polar spindle fibres. The mutual contact or agglutination of the chromosome staves takes place first in the region of the clump that is nearest to the pole, their more distal portions remaining longer free: so that at this stage we get the image of a compact ring with digitiform processes depending from it—the 'figures pectiniformes' of Henneguy (figs. 32 and 34). In badly fixed cells the clumping results in a formless mass, in which the chromosomes seem to have become completely fused together. This state is shown in fig. 34. But, as I gather from the study of my most favourably fixed specimens, this is an artefact; and there is not at any time a real fusion of the chromosomes, but only intimate contact to the point of indistinctness, or possibly superficial agglutination.[1] Fig. 33 seems to me to show the utmost degree of agglutination that should be taken to be normal; and the real state of things to be fairly well represented by fig. 30 or 31.

Careful examination of the staves of the clump at this stage seems to show that they are always in reality double structures; for in favourable cases they show unmistakable indications of a longitudinal duplicity. In fig. 29 there are four staves, marked with a cross, which show this. In the left-hand one (near the top) the tip is distinctly bifid; and this is

[1] Cf. Janssens, 'La Cellule', xix. 2, 1901, p. 86, and Janssens et Dumez, ibid., xx. 2, 1903, p. 450 and fig. 15, who have arrived at the same conclusion.

also the case with the one at the bottom. In the two right-hand ones the tips are distinctly double ; and by careful focusing it can be made out that each of these staves is composed of two longitudinal moieties, superposed and to a slight extent twisted round one another. And in three or four of the short dark staves of the inner tier there can be seen a light longitudinal dividing line (not sufficiently clear in the drawing).

In fig. 33 nearly one-half of the twenty-one staves drawn are seen to be notched at the periphery, and two of them show a longitudinal dividing line continuing the notch inwards. In fig. 30 I find three cases similar to these, and in fig. 32 two. I have no doubt that with better fixation these nuclei would have shown several more such cases. In the clump of fig. 31 I think I can detect three or four similar cases, though doubtfully.

The clump does not long remain in this state of dense agglomeration, but soon begins to expand into the telophasic ring. The manner of this expansion is as follows. Amongst the staves of the clump—but never on their outer surfaces—there appear certain hyaline globules which, growing, push the staves apart and so loosen the clump. In fig. 38 are shown two such globules, one to the right, and one to the left ; in fig. 35 three (on the left ; one very indistinct) ; in fig. 37 five ; in the nucleus of fig. 36 there are a dozen or so, of which only a portion of one (at the left) could be shown in the drawing, the rest being too much masked by the sheaths. In fig. 62, to the right, are seen three ; in fig. 67 two can just be glimpsed (at the left and middle). These globules are entirely hyaline and uncolourable. Their outlines are generally quite smooth. They are, as I think, ovoid in shape, not spherical : they may show a circular outline, as in the left-hand ones of figs. 38 and 43, and other places ; but that is the expression of a transverse section of them. I suspect that there is formed at first one of them for each chromosome. If that be the case it is a likely hypothesis that they consist of the clear contents of the sheaths of the chromosomes, expressed from them by the pressure of the clump. But it is difficult to ascertain the number formed, because they soon fuse with one another into a small number of large globules, see figs. 43, 44, 45.

They ultimately all fuse, apparently, into a single homogeneous ring, as shown in figs. 49, 50, and others.

As soon as these globules have attained a certain size, figs. 43, 44, 45, 49, and less clearly yet still indubitably in figs. 36, 37, 38, the chromosomes, which in the clump appear as straight staves, now appear as more or less sharply curved staves, set on the surface of the globules or ring, that is, outside them and not embedded in them, see particularly the profile views figs. 43, 44, 45. Their outer surface is irregularly convex; but their inner surface is flattened on to the curvature of the globule or ring. They are—at the stage we are considering—of a length equal to about that of one of the limbs of the V-shaped chromosomes of the anaphase (see figs. 3, 4, 17, 61). They do not form complete hoops round the ring, but arcs that embrace about half a meridian of it. They thus show two ends, a polar end and an antipolar end. The polar ends, abutting on the lumen of the ring, are generally closely huddled together and sharply curved downwards, so that it is impossible to get clear images of them. But their antipolar ends are generally widely spaced (figs. 43, 44, 45), and here their two component threads may frequently be seen, with certainty, to be widely divaricated, figs. 43 (in the middle), 44, 45, which is not the case with the polar ends.

As soon as the process of expansion has set in, the images of the clump become less indistinct, and the chromosome staves appear as shown in figs. 30, 33, 35, 36, 37; that is, they are seen with certainty to contain or consist of the thin chromatic threads running in pairs, which in our study of the clump in Part I we recognized by their structure as shrunken chromosome axes, without discussing the fact of their collocation in pairs. The members of these pairs run very close together and in the main parallel to one another, as shown in figs. 30 to 35. Images such as these may suggest, strongly, that during the earlier stages of the clump the chromosomes have contracted into short staves, each of which has undergone a longitudinal division; so that the threads would be the cleavage products of such a division. Now there is no sign of any such division taking place at any time ; but there is evidence that each of these threads represents an entire limb of the anaphase V from which it is derived ; and that their parallelism in pairs is brought about by the folding together of the two limbs of that V. This evidence is contained in the following considerations.

In the daughter-star of the anaphase the chromosomes are indubitably V-shaped, with equal limbs diverging to an angle of some 45 degrees,[1] figs. 3 and 5 (the apparent shortness of some of the limbs in these figures, and the apparent hook shape, is due partly to unequal degrees of contraction, partly to foreshortening). But as the star passes into the clump stage this divergence becomes less pronounced, and in the completed clump we find no such open V's, but in their place a bundle of short straight staves, figs, 29 to 33, each of which shows the two thin chromatic threads mentioned above. The observer's first impression naturally is that each of these staves represents one limb of a V, the relation of this one to the other being masked by the crowding of the elements. But consideration shows that this can hardly be. For the staves are only present in a far smaller number than the limbs of the anaphase V's—in the completed clump in only half that of the limbs. Take for instance fig. 29. This clump, a very early one, contains, as I make it, thirty-two seeming staves, of which twenty-nine are shown in the drawing. Now the anaphases of Salamandra atra, from which this is taken, have twenty-four V's, therefore forty-eight limbs. Manifestly, therefore, not all the staves of the clump can represent single limbs ; but some of them must represent entire chromosomes. Let us suppose that sixteen of them are in this case ; then these will account for thirty-two limbs ; and the remaining sixteen staves will represent sixteen single limbs, thus making up the required tale of forty-eight. Now take fig. 30, a completed clump. I make out twenty staves shown fairly distinctly (not all drawn), and the unanalysable portions of the clump may account for a very

[1] This for the nuclei of the Amphibia. As we shall see, it is not the case for those of all groups of animals.

few more. So here we have about twenty-four staves, representing forty-eight limbs. Or take fig. 33, also a completed clump. It shows twenty-one staves, and may contain a very few more. Therefore here again about twenty-four staves for forty-eight original limbs. Now take fig. 31, a nearly completed clump from Bombinator igneus. The diploid number of chromosomes in this species is sixteen, showing therefore thirty-two limbs at the anaphase. The clump contains twenty staves. Therefore not all of these can represent limbs of V's; but twelve of them probably represent twelve whole V's, and the remaining eight represent single limbs of such: total, thirty-two.

It is therefore certain that in any polar clump some of the staves—and highly probable that in the completed clump all of the staves—must represent each of them two limbs of a V. And the conclusion follows, that each of those of the completed clump is in fact a V whose limbs have folded together. So that the observed duplicity of the staves is not due to the chromosomes having undergone a cleavage after having in some other way assumed the shape of staves, but to their consisting of the two limbs of an anaphase V—or what remains of these. For the folding fully accounts for the duplicity.

In the Amphibia the postulated folding of the V's takes place as a rule only during the formation of the polar clump, not before. But exceptionally it may take place during the early anaphase. Fig. 4 is a case in point. In this anaphase the limbs of the V's are in several instances closed in to a distance of only about half a micron (as measured by the drumhead of the fine-adjustment), and so accurately superposed on radii of the figure that it is only by the most careful attention that the elements can be seen to consist of two superposed moieties.

But this, which in the Amphibia seems to be the exception, is in some other animal groups the invariable rule. For instance, in the spermatogonia of the Acridian Oedipoda cothurna (Arcyoptera variegata) I invariably find the state of things represented in fig. 61. This is a sagittal section of a mid-anaphase, the chromosomes being not yet half-way to the pole. They consist, all of them, of tightly-folded V's, appearing as short staves with the spindle-fibre insertion at the end. But they are certainly folded V's with the insertion at the apex: the two limbs can be made out with certainty at the tips of four of them; and a longitudinal duplicity can be at least glimpsed in all of them.[1] I find the same state of things exactly in Oedipoda germanica, Oe. coerulescens, Oe. (Mecostethus) parapleura, Gomphocerus rufus, Stenobothrus morio, St. biguttulus, and some other species of Stenobothrus which could not be determined with certainty. So that in all the Acrididae I have examined the folding takes place not later than the early anaphase. And as at this stage the images are not obscured by the crowding of the chromosomes which takes place in the polar clump, there can be no doubt about the folding actually occurring.

So also in the Locustidae. Fig. 64 shows an anaphase of a spermatogonium of Decticus verrucivorus. The chromosomes are here smaller than in the Acrididae, and appear for the most part as short rods with the spindle-insertion at the end. But it can be made out in favourable instances that they are in reality folded V's; and where this cannot be done, the analogy with those of the Acrididae puts it out of doubt that they are in the same case. Similar images are afforded by Decticus griseus, Locusta viridissima, L. cantans, and Pterolepis aptera. In Gryllotalpa vulgaris and Gryllus campestris I find apparently the same state of things, the anaphase chromosomes (with the exception of the monosome in Gryllus) appearing as short rods inserted by one end on the spindle. These apparent rods are too small to be analysed with certainty; but judging by the analogy of those of the other Orthoptera mentioned there can be no doubt that they are in reality

[1] The drawings figs. 12 and 13 (Dissosteira carolina), and 18 (Steiroxys), of the paper of Davis, "Spermatogenesis in Acrididae", in 'Bull. Mus. Comp. Zool. Harvard', with the interpretations given, pp. 69, 70, 71 of the text, should, as I conceive, be corrected in the sense indicated above.

tightly-folded V's.[1] And this is also doubtless the case with the very short thick chromosomes of the Hemipteron Pentatoma (Carpocoris) nigricornis.

We find, then, that in the nuclei we have been studying the chromosomes become doubled at the telophase, or before, through a folding-in of their limbs. This brings those limbs into a state of parasyndesis or close juxtaposition throughout their length, so that little change (other than the elongation due to their growth during the interphase) is required in order to bring them into the state in which they are found at the commencement of the spireme stage. This is illustrated in figs. 55 to 59. But this process is perhaps not followed exactly in all nuclei. I have evidence that the folding, or at all events the definitive parasyndesis, of the limbs may be deferred, and

[1] In the Orthoptera the folding takes place not only as early as the early anaphase, but sometimes as early as the equatorial phase. In the equatorial figures shown in figs. 60 (Oedipoda cothurna) and 63 (Decticus verrucivorus) all the chromosomes are tightly folded into the stave shape. The same is the case in Oedipoda germanica, Oe. coerulescens, and Oe. (Mecostethus) parapleura. In Gomphocerus rufus the majority of the chromosomes appear in the stave form; but there may be some open V's. In Stenobothrus biguttulus I suspect that the equatorials have always exactly two large chromosomes of the open V shape, all the others being tightly folded into the stave shape. It is perhaps not rash to conclude that all the cases of chromosomes described by authors as straight rods with a terminal spindle insertion are in reality cases of tightly-folded V's with an apical spindle insertion.

Fig. 63 (Decticus verrucivorus) shows sixteen large autosomes, fourteen small ones, and a monosome, therefore thirty-one in all. This is as it should be: for in this species I find in all unobjectionable images either sixteen large autosomes and fourteen small, or fifteen large and fifteen small, and a monosome; the difference resulting from the fact that it is sometimes difficult to decide whether a chromosome is an unusually small 'large' one or an unusually large 'small' one. Buchner ('Arch. Zellforsch.', iii. p. 342, and fig. 82 of Taf. xix) correctly gives the number as thirty-one in all. Vejdovsky (op. cit., pp. 33 and 44), notwithstanding that he had this description before him, insists that there are only twenty-three in all. Reference to his figs. 65 to 69 shows that he has mistaken entire chromosomes tightly folded into the stave shape, and fortuitously approximated at their apices, for mere limbs of open V's.

take place only at the moment of the formation of the spireme, or even at an advanced period of its evolution. In this case, the limbs pass through the interphase in a more or less widely divaricated state, which gives to the interphase a facies very dissimilar to that of the interphase of nuclei in which the parasyndesis has taken place at the telophase. A description of this is reserved for a future paper. But in either case the mechanism of the division of the chromosomes is the same in principle. *There is no longitudinal splitting.* The division is a *transverse* one, brought about by the folding of the chromosomes at their middle, and their ultimate segmentation at the bend there formed. The moieties which separate at the metaphase are *the two limbs* of the chromosome thus folded, therefore metameric, not antimeric, moieties

EXPLANATION OF PLATES 1 AND 2.

Illustrating Mr. Arthur Bolles Lee's paper on 'The Structure of certain Chromosomes, and the Mechanism of their Division'.

Magnification 1,500 diameters throughout.

PLATE 1.

Fig. 1.—Anaphase of pollen grain of *Paris quadrifolia*. Chromosomes alveolated, with sheath.

Fig. 2.—Early interphase of pollen grain of P. *quadrifolia*. Chromosomes without sheath, not alveolated, elongated into spirals.

Fig. 3.—*Triton alpestris*. Anaphase of spermatogonium. The chromosomes as open V's, showing the chromatic axis and periaxial spirals and sheath.

Fig. 4.—The same, a somewhat later stage, showing the chromosomes folded into very narrow V's.

Fig. 5.—*Bombinator igneus*, spermatogonium. Portion of anaphase, showing the chromosome axes and periaxial spirals, but not the sheath.

Fig. 6.—*Salamandra maculosa*. One limb of an anaphase chromosome, spermatogonium. Chromatic axis, periaxial spirals (very widely spaced), lateral processes, and sheath.

Fig. 7.—Salamandra atra, do., do. Shows same details; also the terminal tag on the dome-shaped end of the axis.

Fig. 8.—Salamandra maculosa, oogonium. Anaphase chromosome, entire. Same details.

Fig. 9.—Do., epiderm. Anaphase chromosome, one limb. Same details.

Fig. 10.—Do., epidermal gland; anaphase; one limb of a chromosome. Spiral with very wide pitch.

Fig. 11.—Do., kidney cell. Same details.

Fig. 12.—Do., cornea. Spirals much flattened on to axis.

Fig. 13.—Do., retina of larva, rod and cone layer. Details as last.

Fig. 14.—Triton alpestris, larva, pulmonary epithelium. Entire anaphase chromosome. Note the spiral very closely coiled at tip of right-hand limb, and not continued round the polar bend.

Fig. 15.—*a*, Triton palmatus, spermatogonium; *b*, Salamandra maculosa, spermatogonium; *c*, do., epiderm. Transverse sections of anaphase spermatogonia. See text.

Fig. 16.—Homo, pus corpuscle from ulcerated skin. Two chromosomes from an equatorial division figure. Sheath and lateral processes shown, periaxial spirals invisible, though doubtless existent.

Fig. 17.—Gallus domesticus, embryonic cartilage. Portion of an anaphase. Periaxial spirals just visible, sheath strong.

Fig. 18.—Ancylus lacustris, buccal epithelium. Tangential section of anaphase. Spirals, lateral processes, and sheath just visible.

Fig. 19.—Salamandra maculosa, epiderm. Chromosome from a not completely regularized equatorial figure. Spirals indistinct, giving an impression of 'granules'.

Fig. 20.—Do., from a completed equatorial figure of a spermatogonium. Details as last.

Fig. 21.—Do., portion of equatorial chromosome of an oogonium. Details as last two figs.

Fig. 22.—Do., renal epithelium. One limb of an equatorial chromosome. Spirals distinct on each of the two moieties.

Fig. 23.—Oedipoda cothurna. Equatorial chromosome of secondary spermatogonium. Details as for fig. 19, but sheath stronger.

Fig. 24.—Triton palmatus, spermatogonium. Spireme, early stage. Chromosomes double, no sheath or other detail.

Fig. 25.—Salamandra maculosa, larva, epithelium. Spireme somewhat more advanced than last. Moieties of chromosomes varicose (dawn of periaxial spirals).

Fig. 26.—Do., pulmonary epithelium. Spireme, later stage. Moieties very varicose, with abundant lateral processes and sheath.

Fig. 27.—Do., pleural endothelium. 'Segmented' spireme. Moieties with large varicosities (Pfitzner's 'granules'), and lateral processes and sheath.

Fig. 28.—Triton palmatus, spermatogonium. Later spireme. Periaxial spirals can just be glimpsed.

Fig. 29.—Salamandra atra. Spermatogonium. End of anaphase. Chromosome V's folded into the stave form.

Fig. 30.—Triton palmatus. Spermatogonium. Polar clump. Chromosomes tightly folded, much contracted.

Fig. 31.—Bombinator igneus. Spermatogonium. Polar clump. As last.

Fig. 32.—Triton palmatus. Spermatogonium. Clump showing chromosomes coalesced. Wholly or in part an artefact.

Fig. 33.—Do., do., do. Clump in polar view.

Fig. 34.—Triton alpestris. Do., do., do. Profile view.

Fig. 35.—Triton palmatus. Do. Clump expanding, early stage.

Fig. 36.—Do., do., do. Later stage.

Fig. 37.—Do., do., do. Later stage of expansion, clump passing into telophase.

Fig. 38.—Do., do., do. Same stage, profile view.

Fig. 39.—Salamandra maculosa. Oogonium. Same stage, or early telophase. Axes of limbs of chromosomes closely entwined round one another.

Fig. 40.—Do., do., do. Somewhat later stage, chromosomes elongating.

Fig. 41.—Bombinator igneus, spermatogonium. Clump in stage of figs. 38 and 39.

Plate 2.

Fig. 42.—Salamandra maculosa, spermatogonium. Telophase, early, showing telophasic ring in profile (section).

Fig. 43.—Triton palmatus. Do., do., do. Note the chromosomes flattened on to the outside of the hyaline globules, which are in course of fusing into a ring.

Fig. 44.—Do., do., do. Tangential section of ring. As last. Two large hyaline globules shown in the middle. Note the ends of the chromosome axes showing divaricated at the antipolar ends.

Fig. 45.—Do., do. Profile view of a ring at a slightly later stage. Chromosome moieties looser; chromosomes longer.

Fig. 46.—Salamandra maculosa. Renal epithelium. Telophasic ring, same stage as last, same details.

Fig. 47.—Bombinator igneus. Spermatogonium. Section of ring, same stage as last, and same details.

Fig. 48.—Do., do. Later stage of telophasic ring, polar view.

Fig. 49.—Triton palmatus. Polymorph spermatogonium. Mid-telophase, ring beginning to close. Chromosomes elongated.

Fig. 50.—Salamandra maculosa, oogonium (primary). Telophasic ring, about same stage as last, chromosomes more elongated and taking on an erratic course.

Fig. 51.—Triton palmatus. Large endothelium nucleus from follicle of testis. Late telophase, ring almost closed. Nucleus very flat; almost all the chromosomes drawn; chromosome axes distinctly doubled and entwined.

Fig. 52.—Do., do., a smaller nucleus, somewhat later stage.

Fig. 53.—Bombinator igneus. Endothelium nucleus, entire, testicular peritoneum. Polar view (not a section) of telophase of same stage as last. All the chromosomes have been drawn, though not throughout all their length.

Fig. 54.—Homo. Endothelium of vein of cutis. Section of telophase, about the stage of fig. 51 or 53.

Fig. 55.—Triton palmatus. Spermatogonium, early interphase.

Fig. 56.—Do. Late interphase, or dawn of spireme.

Fig. 57.—Do., do. Early spireme. Karyoplasm browned by osmium.

Fig. 58.—Bombinator igneus. Peritoneal endothelium. Early rest stage.

Fig. 59.—Do., do. Later rest stage.

Fig. 60.—Oedipoda cothurna. Spermatogonium. One half of an equatorial figure. Chromosomes all of them as tightly-folded V's.

Fig. 61.—Do., do. Sagittal section of anaphase. Chromosomes so tightly folded that they appear as stout curved staves.

Fig. 62.—Do., do. Early telophase, tangential section of ring. Shows three hyaline globules (to the right).

Fig. 63.—Decticus verrucivorus. Spermatogonium. Equatorial figure. All the chromosomes drawn. All are tightly folded into the stave shape; *m* is the monosome.

Fig. 64.—Do., do, anaphase, polar view. Chromosomes folded into the shape of wedges; *m*, monosome.

Fig. 65.—Do., do. End of anaphase. Chromosomes as before.

Fig. 66.—Do. Primary spermatogonium. Mid-telophase· *m*, the monosome. Some of the chromosomes seem to have their moieties divaricated at both ends, as if a transverse segmentation had taken place at the polar ends.

Fig. 67.—Do. Nucleus of connective tissue enclosing cyst of testis. Early telophase.